High-performance liquid chromatographic analysis of *Solanum* steroidal alkaloids

Solanum species contain as major secondary metabolites, steroidal alkaloids usually as glycosides (generally referred to as glycoalkaloids). Earlier interest centered on the use of these compounds as starting material for synthetic production of steroidal hormones[1]; however, more recent interest has been on the role of these compounds in pest resistance and culinary quality of edible Solanaceous crops such as potato[2]. In the development of new hybrid strains it is desireable to know the alkaloid compositions of the wild species parents and their hybrids so that new alkaloids are not inadvertently introduced into our food crops. There are many methods that have been described for glycoalkaloid analysis using high-performance liquid chromatography (HPLC)[3–5] thin-layer chromatography (TLC)[6] or gas chromatography (GC)[7]; however, all of these techniques have significant limitations which are mainly due to the hydrophobic (aglycone)–hydrophilic (carbohydrate) dicotomous nature of the glycoalkaloid structure. HPLC has been particularly useful in the analysis of potato glycoalkaloid mixtures. However, separations by HPLC depend, for the most part, on the structure of the carbohydrate portion of the molecule. At this time only glycoalkaloids that have gross differences in aglycone structure can be separated by HPLC when they contain glycosidic units of similar polarity.

We have recently been examining the glycoalkaloid composition of some disease and pest resistant somatic hybrids obtained by protoplast fusion. The glycoalkaloids in these hybrids have similar structures which we were unable to separate by available methods. For analysis of these hybrids, we have concentrated on analyzing the aglycones (*i.e.*, steroidal alkaloids) rather than attempting to separate the glycoalkaloids in the mixture.

For the analysis of somatic hybrids between *S. brevidens* and *S. tuberosum*, the separation of saturated from Δ^5 (5,6 unsaturated) alkaloids presented an especially difficult chromatographic challenge. Methods described for the analysis of the steroidal alkaloids by GC[8], HPLC[9,10] and TLC[11] were of limited use because of poor resolution and/or low sensitivity of detection. In this paper we describe a method for

the separation and quantitation of *Solanum* steroidal alkaloids by reversed-phase HPLC which is particularly useful for the separation of saturated from unsaturated alkaloids.

EXPERIMENTAL

Materials

Solanidine, demissidine, tomatidine and solasodine were purchased from Sigma (St. Louis MO, U.S.A.), leptinidine (23-hydroxysolanidine), acetylleptinidine, 23-hydroxy- and 23-acetyldemissidine and 5-tomatidenol were isolated from plant tissue as the glycosides and partially purified by ammonia precipitation. The glycosides were hydrolyzed in 1 *M* methanolic HCl. The individual aglycones were isolated by preparative TLC on silica gel using appropriate solvent systems[11]. The purified aglycones were characterized by either gas chromatography– or direct probe–mass spectrometry.

Alkaloid mixtures from *Solanum* hybrids were prepared by hydrolysis of the glycoalkaloid mixture with 1 *M* methanolic HCl.

All solvents were high-purity grade from Burdick & Jackson, (St. Louis, MO, U.S.A.).

Chromatographic procedure

HPLC analyses were carried out on a Hewlett-Packard 1090 instrument fitted with a Supelcosil LC-18-DB (5 μm) column (25 cm $\times$ 4.6 mm I.D.) (Supelco, Bellefonte, PA, U.S.A.) and a Model 1037A refractive index (RI) detector (Hewlett-Packard). The mobile phase was acetonitrile –methanol–ethanolamine (60:40:0.001) and the flow-rate was 0.5 ml min^{-1}.

RESULTS AND DISCUSSION

The HPLC chromatogram for the steroidal alkaloids shown in Fig. 1 is given in Fig. 2. These nine steroidal alkaloids are the major aglycones of the *Solanum*

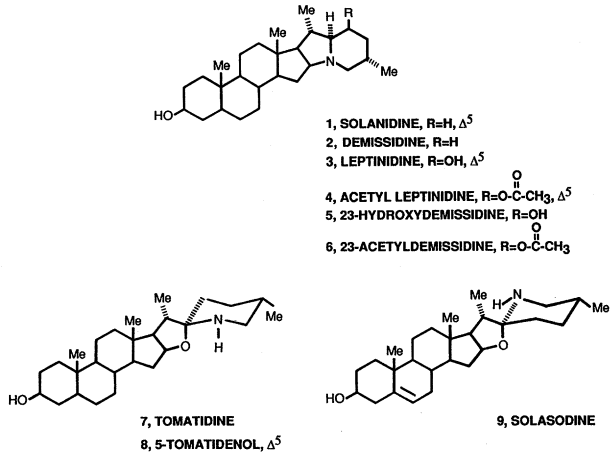

Fig. 1. Structures of *Solanum* steroidal alkaloids. Me = Methyl.

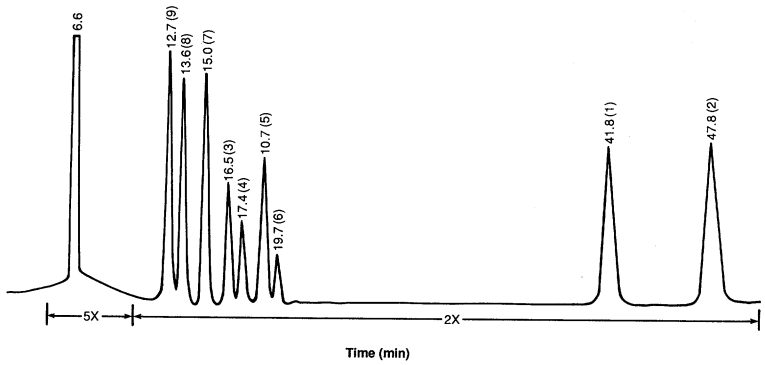

Fig. 2. Reversed-phase HPLC of *Solanum* steroidal alkaloids. For conditions, see Experimental.

glycoalkaloids[12] as well as glycoalkaloids that have only been found in experimental hybrids[13]. Other alkaloid aglycones have been reported[12], however, these rare aglycones were not available to us.

Although partial separation of demissidine and its Δ^5 analogue, solanidine has been achieved by capillary GC[8], other such analogues cannot be separated under the same conditions. The alkaloids, as is the case observed with steroids in general, decompose at the high temperatures required in the GC analysis which adversely effects sensitivity, quantitation (we have been able to detect lower concentrations of solanidine which elutes at 240°C than tomatidine which elutes at 270°C) and resolution.

One objection to the use of HPLC for analysis of the steroidal alkaloids has been the poor sensitivity of available detection devices. Generally, these compounds exhibit no appreciable absorption above 200 nm which limits the use of UV detection. Tomatidine and 5-tomatidenol have been separated by normal-phase HPLC with UV detection; however, 210 μg of tomatidine was injected and base line separation was not achieved[9]. Until recently, detectors were not sufficiently sensitive to use for the level of detection required in analysis of alkaloid extracts, however, RI detectors are now available that can detect these compounds at concentration levels well below that required in most, if not all, analyses. Using tomatidine as a representative alkaloid, we have determined that one can detect quantities as low as 200 ng which is approximately a thousand fold increase in sensitivity over that reported for UV detection without the problem of UV variable response for different chromophores.

The HPLC analysis of the steroidal alkaloid fraction of a *Solanum* hybrid[14] (supplied to us by Dr. J. P. Helgeson, Agricultural Research Service, U.S. Department of Agriculture, University Wisconsin, U.S.A.) which contains Δ^5 and saturated analogues is shown in Fig. 3a. For comparison purposes, the GC for this same mixture is shown in Fig. 3b. HPLC analysis of this mixture revealed two components (22 and 24.5 min) which were not readily apparent by other chromatographic procedures. It was established by mass spectrometry on collected samples of these peaks that the compounds are hydroxysolanidanes, the former being unsaturated and the latter the saturated compound. However, on the basis of retention (see Fig. 2) and mass spectral data, these compounds are not 23-hydroxysolanidine or 23-hydroxydemissidine. The superior separation of the saturate/Δ^5 pairs compared to GC is evident.

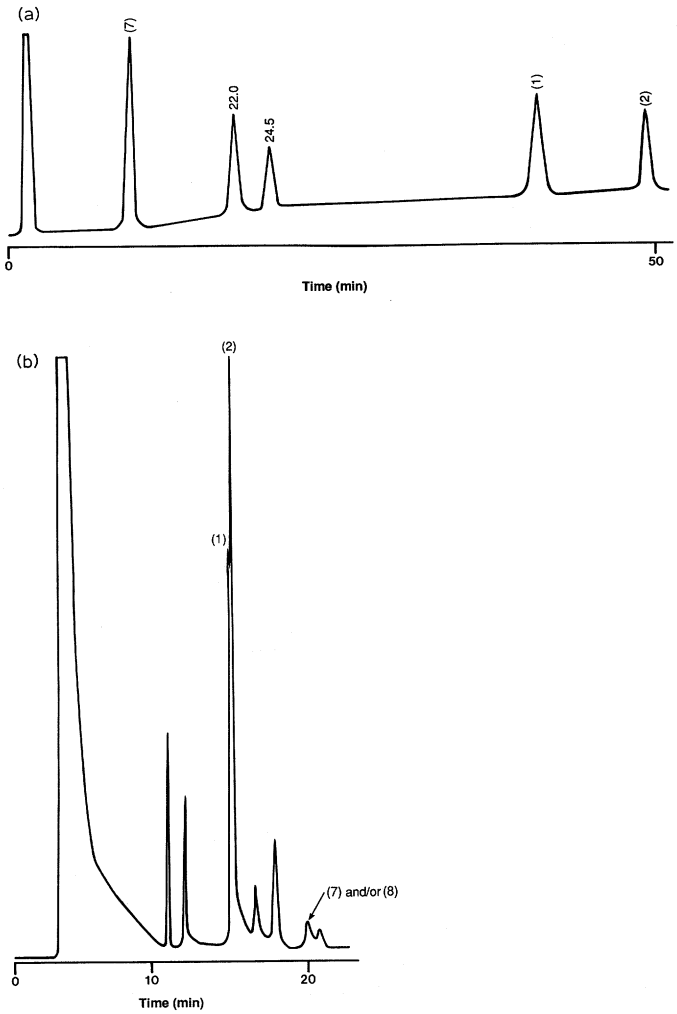

Fig. 3. Analysis of alkaloid fraction of *S. brevidens* × *S. tuberosum* hybrid. (a) HPLC chromatogram; (b) GC chromatogram. For conditions, see ref. 8.

The chromatogram of the tuber alkaloids of the commercial potato variety, Kahtadin, is shown in Fig. 4. Although there are some minor unidentified compounds in this alkaloid extract, one alkaloid, solanidine represents at least 90% of the sample.

Of all the reported methods for separating *Solanum* steroid alkaloids, in our estimation, this HPLC method provides the best separation of saturated and Δ^5 analogues (*e.g.*, solanidine and demissidine). Chromatographic conditions can be varied depending on the composition of the alkaloid mixture. On the basis of preliminary results we have for the separation of partially characterized dihydroxy steroidal alkaloids using a solvent mixture of acetonitrile–methanol (85:15) at a flow-rate of 0.5 ml min^{-1} provides better resolution of the solvent peaks from the

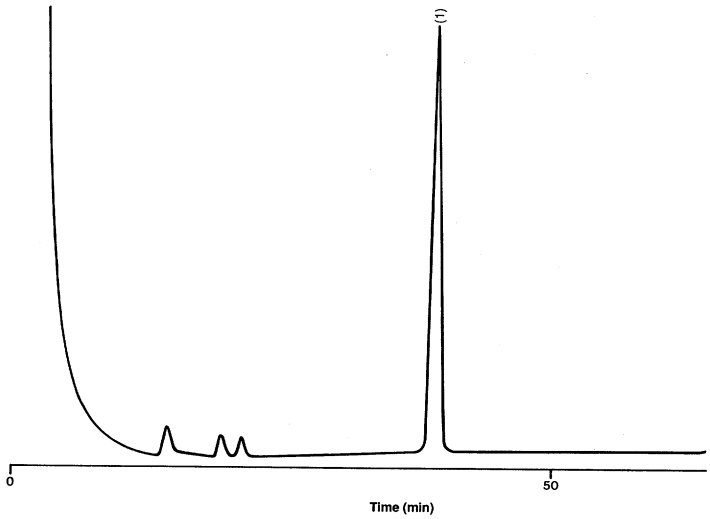

Fig. 4. HPLC chromatogram of the alkaloid fraction from the cultivated potato variety, Kathadin.

compounds of interest. If the alkaloid mixture contains compounds that are in the order of polarity or less polar than solasodine the 60:40 solvent mixture at a flow-rate of 1 ml min^{-1} results in shorter analysis time without significant loss of resolution. It is necessary in all solvent systems to include ethanolamine even when using the DB-modified column in order to obtain good elution profiles for these alkaloids.

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